

Figure S1. related to Figure 1. Langerhans cells are necessary and sufficient for Th17 responses to C. albicans.

(A) Human Langerin-DTR mice were adoptively transferred with 3x10⁵ TEα cells and treated the next day either with PBS or 1μg diphtheria toxin i.p. to selectively deplete Langerhans cells. Four days later mice were epicutaneously infected with 2x10⁸ CFU of Eno1-Ag. T cells were isolated from skin draining lymph nodes and stimulated with PMA/Ionomycin. Expansion (top row) and cytokine production as determined by intracellular flow cytometry is shown (bottom row). (B) WT or Human Langerin-DTR mice received 3x10⁵ TEα cells. One day later mice were immunized with 1 μg 2G3-Eα ip. Six hours later, they were either mock or SC5314 epicutaneously infected. Four days later, T cells were isolated from skin draining lymph nodes and stimulated with PMA/Ionomycin. Expansion (top row) and cytokine production as determined by intracellular flow cytometry (bottom row) is shown. Representative data from 3 experiments is shown.

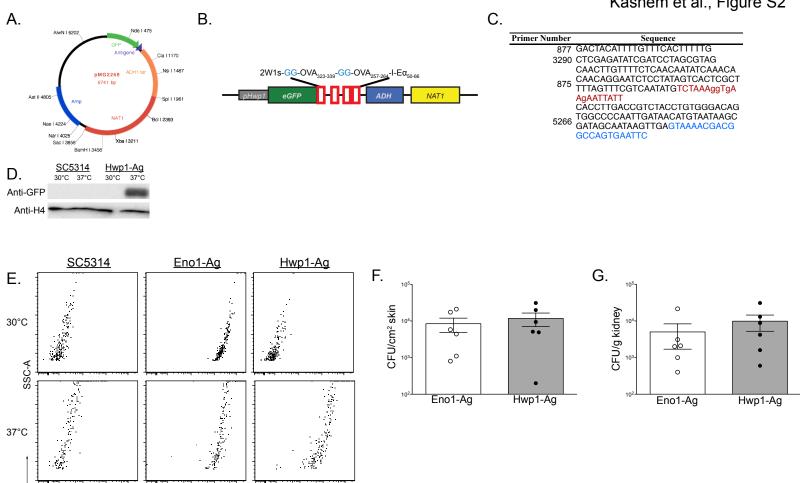
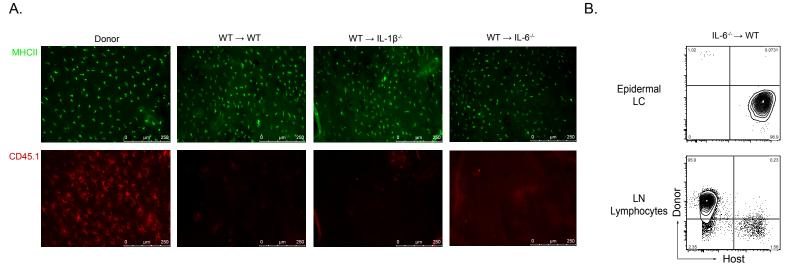


Figure S2., related to Figure 1. Generation of Hwp1-Ag.

(A) Design of vector inserting GFP-Ag cassette behind the hyphal wall protein 1 promoter. (B) Schematic drawing of Hwp-1Ag. (C) Primers used in design of Hwp1-Ag. (D) Western blot showing GFP and control H4 expression in Eno1-Ag compared to Hwp1-Ag under different morphological growth conditions. (E) GFP expression as demonstrated by flow cytometry of SC5314, Eno1-Ag and Hwp1-Ag under yeast (top) and filamentous growth conditions (bottom). (F) WT mice were infected epicutaneously with 2x108 Eno1-Ag or Hwp1-Ag. CFU from 1 cm2 skin sections harvested 3 days after infection from the central back are shown. (G) WT mice were i.v. infected with 1x105 Eno1-Ag or Hwp1-Ag. CFU of kidneys harvested 3 days after infection are shown. Symbols represent data from individual mice.



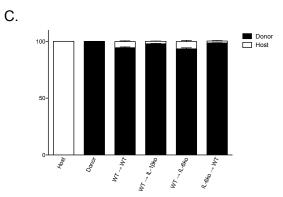


Figure S3., related to Figure 3. Efficacy of chimerism post bone marrow transplant. Six weeks old knock-out, C57BL/6 or Ly5.2 congenically marked mice were irradiated with split doses of 500 cGy. The following day, 5x10⁶ bone marrow cells isolated from the specified mice were injected intravenously. Mice were rested for at least 6 weeks prior to experiments. Chimerism was assessed by expression of CD45.1 and CD45.2 by LC in epidermis as well as CD45+ lymphocytes in blood and lymph nodes. (A) Demonstration of the persistence of host radio-resistant CD45.2 LC in recipient skin. (B) The percentage of host (CD45.2) and donor (CD45.1) skin LC (top panel) and total LN lymphocytes (bottom) in *II6*^{-/-}→WT bone marrow chimeric mice is shown. (C) The relative degree of chimerism in the lymph nodes of the indicated chimeras based on expression of CD45.1 and CD45.2 is shown.

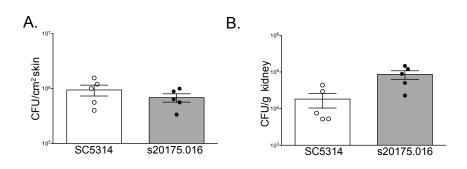


Figure S4, related to figure 4. SC5314 and s20175.016 have similar virulence.

(A) WT mice were intradermally infected with 2x10⁸ SC5314 or s20175.016. Mice were euthanized 3 days later and 1.0 cm² skin sections harvested from central back of mice. The total CFU is shown. B) WT mice were i.v. infected with 1x10⁵ SC5314 or s20175.016. The CFU in kidneys isolated 2 days after infection is shown. Each symbol represents an individual animal.

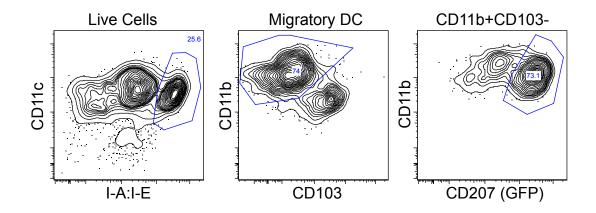


Figure S5, related to Figure 4. Sorting strategy for epidermal LC in the draining lymph nodes.

(A) Groups of Langerin-eGFP mice were either mock, SC5314 or s20175.016 epicutaneously infected. 3 days later DC were purified from skin draining lymph nodes by CD11c+ magnetic bead selection and sorted on live, singlets. LC were identified as CD11c hi, I-A/I-E hi, CD11b hi, CD103 negative, GFP positive cells.

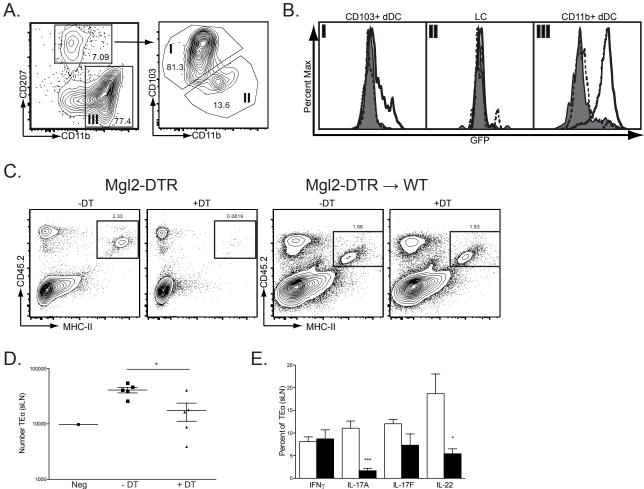


Figure S6, related to Figure 5. Mgl2-DTR mice have depletion of LC and reduced Th17 in response to C. albicans infection.

Dendritic cells were isolated from skin draining lymph nodes and gated as singlet, live, MHC-II hi, CD11c hi cells. (A) DC were further gating using CD103, CD11b and CD207 to identify 3 groups (I-CD103+ ddC, II-LC, III-CD11b+ ddC). (B) Expression of MgI2 in the indicated DC populations as indicated by expression GFP in WT (gray filled), or MgI2-DTR WT mice 1 day after injection of vehicle (black line) or DT (dotted line). (C) Single cell epidermal suspensions were stained with MHC-II and CD45.2 to identify the percentage of LC in the epidermis of MgI2-DTR (left) or MgI2-DTR WT (right) mice one day after treatment with vehicle or DT. (D) MgI2-DTR mice were transplanted with 3x10⁵ TEα cells and treated with PBS (white) or DT (black). On day +1, mice were either mock infected of epicutaneously infected with Eno1-Ag. Expansion and (E) cytokine production of PMA/Ionomycin stimulated CD90.1+ TEα cells in MgI2-DTR mice treated with PBS (white) or DT (black) is shown.

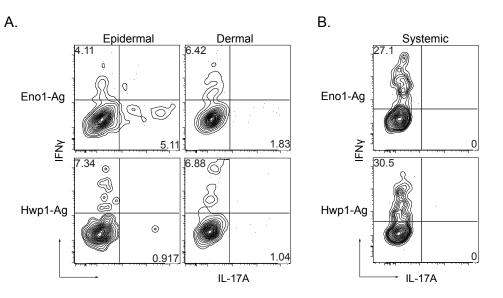


Figure S7, related to Figure 6. Th17 is generated only after epicutaneous infection with Eno1-Ag.

(A) Mice adoptively transferred with $TE\alpha$ cells were infected epicutaneously (left) or dermally (right) with either $2x10^8$ Eno-1Ag (top panels) or Hwp-1Ag (bottom panels). Four days later, T cells were isolated from lymph nodes and stimulated with PMA and lonomycin. The expression of IL-17 and IFNy production by CD90.1 $TE\alpha$ cells is shown. (B) as in (A) except mice were i.v. infected with 10^5 Eno1-Ag or HWP1-Ag. Data are representative of 3 experiments.